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1. Results of intraperitoneal (IP) administration of $PtCl_4$ or $Pd(NO_3)_2$ are reported. Administration at levels of 28 or 56 moles/kg body weight decreased the thymidine incorporation into DNA of spleen, liver, and testis.

2. Effects of various salts of platinum or palladium administered by intraperitoneal injection or ingestion were determined on the parameters of the microsomal mixed function oxidase system from rat liver.

3. Lethal-dose studies are reported following the intraperitoneal or oral administration of salts of lead, manganese, platinum, and palladium to young male rats. Studies have been conducted on the effect of dietary administration of salts of Pb, Mn, Pt, and Pd on the following: the growth rate of male rats, the organ weight of five tissues (liver, kidney, spleen, heart, and testis), and the tissue content of DNA, RNA, and protein.

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. ASSESSMENT OF TOXICITY OF AUTOMOTIVE METALLIC EMISSIONS. VOLUME II:

Relative Toxicities of Automotive Metallic Emissions Against Lead Compounds Using Biochemical Parameters

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ABSTRACT

SECTION I

The intraperitoneal administration of $PtCl_4$ or $Pd(NO_3)_2$ at levels of 28 or 56 μmoles/kg body weight decreased the thymidine incorporation into DNA of spleen, liver, kidney and testis. Spleen was most sensitive to both the platinum and the palladium salt. In liver, DNA syntheses in parenchymal cells and stromal cells were about equally sensitive to $PtCl_4$. In control rats, only 20-30% of the ^3H in the acid-soluble fraction of liver or spleen was in the form of thymidine and its phosphate esters 2 hr after the intraperitoneal injection of ^3H-thymidine; prior injection of $PtCl_4$ (56 μmoles/kg body weight) did not change the pattern.

SECTION II

The effects of various salts of platinum or palladium were determined on the parameters of the microsomal mixed-function oxidase system from rat liver. The intraperitoneal injection of $PtCl_4$ or $Pd(NO_3)_2$ at 56 μmoles/kg, increased the hexobarbital-induced sleeping time in vivo and generally decreased the aminopyrine demethylase in vitro and the microsomal content of cytochrome P-450. The dietary administration of various salts of Pt or Pd for one week generally decreased or had no effect on the parameters of drug metabolism by isolated microsomes and after 4 or more weeks generally had no effect on, or increased, the parameters. The addition of 0.15-0.2 mM $PtCl_4$ or 0.2-0.3 mM $Pd(NO_3)_2$ to the incubation medium (containing 5 mM $MgCl_2$) inhibited the aminopyrine demethylase of isolated hepatic microsomes by approximately 50%.

SECTION III

Lethal-dose studies are reported following the intraperitoneal or oral administration of salts of lead, manganese, platinum and palladium to young male rats. Studies have been conducted on the effect of the dietary administration of salts of lead, manganese, platinum and palladium on the following: the growth rate of male rats, the organ weight of five tissues (liver, kidney, spleen, heart and testis), and the tissue content of DNA, RNA and protein.

In general, dietary levels of $PbCl_2$, $PdCl_2$, PdO and $PdSO_4$ greater than 10 mmoles/kg feed were necessary to restrict the weight gain of the rats. Soluble salts of Pt^{4+}, namely $PtCl_4$ and $Pt(SO_4)_2.4H_2O$, at levels of approximately 2 mmoles/liter drinking fluid, were sufficient to restrict the weight gain of treated rats.

Dietary $PbCl_2$ markedly increased the size of kidneys in treated rats. In almost all other studies, however, the dietary administration of salts of lead, manganese, platinum or palladium did not markedly or consisently alter the organ weights of the metal-treated rats. The dietary administration of $PbCl_2$, $PtCl_4$ or $Pt(SO_4)_2.4H_2O$ for 4 weeks did not alter the content of DNA, RNA or protein in liver, kidney or spleen (when the content is expressed per gram of wet tissue).

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SECTION I. EFFECTS OF PLATINUM AND PALLADIUM SALTS ON THYMIDINE INCORPORATION INTO DNA OF RAT TISSUES

INTRODUCTION

Platinum and palladium compounds currently are being used as the active components in the catalytic converters of air pollution control devices on many motor vehicles. It is of interest, therefore, to determine the biological effects of the compounds of these metals. The present experiments were conducted to determine the acute effects of the soluble salts of platinum and palladium on the incorporation of radioactive thymidine into DNA.

MATERIALS AND METHODS

All experimental studies were conducted with male Sprague-Dawley rats (weighing 160-200 g) obtained from Zivic-Miller Laboratories. In each of these experiments, the metallic salt and the ^{3}H-labeled thymidine were injected intraperitoneally 4 hr and 2 hr, respectively, before the removal of the tissues. The ^{3}H-thymidine was injected at a level of 99 μCi/kg body weight, except that a level of 990 μCi/kg body weight was used when the distribution of ^{3}H in the acid soluble fraction was under study (i.e., Table 5). [^{3}H-methyl]thymidine was purchased from New England Nuclear, $Pd(NO_3)_2$ aqueous solution from Research Organic/Inorganic Chemical, and $PtCl_4$ from the latter firm and from B.F Goldsmith Chemical and Metal.

The administered doses of $PtCl_4$ and $Pd(NO_3)_2$ were 14, 28 and 56 μmoles/kg body weight. For $PtCl_4$, these doses corresponded to 2.8, 5.5 and 11 mg Pt^{4+}/kg. For $Pd(NO_3)_2$, the doses by weight were equal to 1.5, 3 and 6 mg Pd^{2+}/kg body weight. The highest dose of Pt, namely 56 μmoles/kg, was equal to one-half of the intraperitoneal LD_{50}. However, because of the steep slope of the lethal dose curve, the dose of 56 μmoles $PtCl_4$/kg was appreciably less than the LD_5.

Thymidine incorporation was measured in spleen, liver, kidney and testis. Tissue macromolecules were precipitated with cold 0.5 M $HClO_4$ and, after centrifugation, the supernatant was collected as the acid-soluble fraction. The RNA in the pellet was hydrolyzed with 0.3 M NaOH (37°; 1 hr) and the intact macromolecules were precipitated with cold $HClO_4$ (final concentration of un-neutralized $HClO_4$, 0.5 M). The DNA was hydrolyzed in hot $HClO_4$ (0.5 M; 90°; 20 min). After centrifugation, the supernatant (hydrolyzed DNA) was collected. The acid-soluble fraction and the hydrolyzed DNA were analyzed for nucleotide concentration by measuring the

absorbance at 260 nm. Tritium was measured by scintillation counting using a mixture containing toluene and Triton X-100 (1).

The radioactivity in the DNA is expressed as counts-per-minute/μmole DNA-nucleotide. The radioactivity in the acid-soluble fraction is expressed as counts-per-minute/μmole total acid-soluble nucleotide. The radioactivity is also expressed as a ratio of the two values, counts-per-minute/μmole DNA-nucleotide divided by counts-per-minute/μmole total acid-soluble nucleotide. This ratio takes into consideration two factors: (a) the total amount of radioactivity available for incorporation into tissue DNA, and (b) any variation in isotope concentration in the same tissue of different rats within an experimental group.

N-1 nuclei from hepatic parenchymal cells and N-2 nuclei from hepatic stromal cells were isolated according to the method of Potter and co-workers (2,3). The method is similar in principle to the method used previously in this laboratory for the fractionation of nuclei from control and regenerating liver (4).

The adsorption of nucleotides and related compounds by charcoal from acidic solution was conducted according to the method of Tsuboi and Price (5). The charcoal-adsorbed compounds were eluted from the charcoal by two treatments with 1% ammonia in 60% ethanol (5). The eluted charcoal-adsorbable compounds were chromatographed on thin layer sheets of polyethyleneimine-cellulose with 0.02 M ammonium acetate-95% ethanol (1:1, v/v). Thymidine and thymine migrated to the solvent front; thymidine phosphates remained at or near the origin.

RESULTS

The effects of $PtCl_4$ on thymidine incorporation into DNA of rat tissues are presented in Table 1. In the tissues studied, the incorporation of thymidine into spleen DNA was the most sensitive to platinum. In spleen, thymidine incorporation was reduced by one-third at the lowest dose of $PtCl_4$. The two higher doses of $PtCl_4$ decreased thymidine incorporation by 50% or more. The ratios (DNA/acid-soluble fraction) gave the same pattern of inhibition. The incorporation of thymidine into liver DNA was not as sensitive to platinum as was the incorporation into spleen DNA. Although no inhibition was observed in liver at a dose of 14 μmoles/kg, thymidine incorporation was inhibited by 40% and the ratio approached that observed in spleen at a dose of 56 μmoles/kg. At the latter dose, the radioactivity in the acid-soluble fraction was increased by approximately 50%. In kidney, thymidine incorporation into DNA was inhibited 40-50% at doses of 28 or 56 μmoles/kg. At the two highest doses, the ratios in kidney approached those obtained in spleen. As in the case of liver, the radioactivity in the acid-soluble fraction was increased by approximately 50% at the highest dose of $PtCl_4$. In testis, the thymidine incorporation into DNA and the ratio were decreased by 25-35% by doses of $PtCl_4$ of 28 or 56 μmoles/kg.

Thymidine incorporation into DNA of spleen was very sensitive to the administration of $Pd(NO_3)_2$, just as it was to $PtCl_4$ (Table 2). Moderate decreases were found in thymidine incorporation into DNA and in the ratio of spleen at the lowest dose of $Pd(NO_3)_2$. At the two highest doses, 50-60% inhibition was observed in the DNA and the ratio. $Pd(NO_3)_2$ decreased incorporation into DNA and the ratio of liver by 25-45%, depending on the

dosage. The kidney was relatively resistant to treatment with $Pd(NO_3)_2$. The maximum inhibition in incorporation in kidney was approximately 40% with a corresponding decrease observed in the ratio. Thymidine incorporation into DNA of testis was markedly inhibited at the two higher doses of $Pd(NO_3)_2$ and the inhibition ranged from 40% to 60%; the decrease in the ratio was comparable at each dose.

The administration of Pt or Pd did not cause a decrease in radioactivity in the acid-soluble fraction in any of the tissues studied (Tables 1 and 2). Thus, the administration of either $PtCl_4$ or $Pd(NO_3)_2$ did not decrease the circulation of the radioactive thymidine from its site of injection and/or the entrance of the thymidine into the tissue. Increases in acid-soluble radioactivity were noted in some experiments. The highest dose of Pt caused this effect in liver, kidney and testis but not in spleen. In contrast, the highest dose of Pd caused an increase only in testis.

Potter and coworkers (2,3) have developed a method for the fractionation of liver nuclei into two classes--nuclei derived from parenchymal cells or hepatocytes (called N-1 nuclei) and nuclei derived from stromal cells or non-hepatocytes (called N-2 nuclei). The effects of $PtCl_4$ on the incorporation of thymidine into DNA of N-1 and N-2 nuclei are presented in Table 3. In experiment A, no inhibition in thymidine incorporation occurred in either class of nuclei and only a small decrease in the ratio was observed at a dose of $PtCl_4$ of 28 μmoles/kg. However, in experiment B, a dose of 56 μmoles Pt/kg decreased thymidine incorporation into DNA to 60% of control values. This dose of $PtCl_4$ also decreased the ratios to approximately 40% of control values. Thus, in experiment B, thymidine incorporation into both N-1 and N-2 nuclei was depressed equally when expressed either as DNA specific activities or as the ratios. Therefore, thymidine incorporation was inhibited to an equal extent in both parenchymal cells and stromal cells.

In control rats, the specific activities of DNA are in the following decreasing order: DNA of N-1 nuclei > total cellular DNA > DNA of N-2 nuclei; the specific activities of DNA from N-2 nuclei are approximately two-thirds those of DNA of N-1 nuclei. In Table 3 (lower portion), the specific activities of DNA from N-2 nuclei are expressed as a fraction of the specific activities of DNA from N-1 nuclei in the same experiment. Although the higher dose of $PtCl_4$ (Table 3) markedly inhibits thymidine incorporation in both cell types, the ratio of the N-2 to N-1 specific activities is not significantly altered by treatment with $PtCl_4$.

The administration of CCl_4 to rats results in the death of some hepatic cells and a rapid DNA synthesis and mitotic activity in surviving cells in order to replace the lost tissue. In data not shown, it was found that thymidine incorporation into DNA is approximately 8-10-fold greater in liver of the CCl_4-treated rats than in the control rats. Incorporation has been studied in four tissues of rats which received CCl_4 and $PtCl_4$ (Table 4). The data for each tissue are compared to the values of animals in group B, which received CCl_4 and a low dose of $PtCl_4$.

In liver, thymidine incorporation into DNA of group B rats was 10-fold greater than that of rats receiving no CCl_4. Moreover, increasing the dose of $PtCl_4$ to 28 μmoles/kg did not inhibit thymidine incorporation into liver DNA of CCl_4-treated rats. For spleen, kidney and testis, thymidine incorporation into DNA of each tissue of groups A and B was essentially equal. In these three tissues, in contrast to the pattern seen in liver, thymidine incorporation in rats of group C (CCl_4 and 28 μmoles $PtCl_4$/kg) was decreased approximately 30% in comparison with group B animals. The same pattern of results was obtained for all

three tissues if the ratios of DNA to acid-soluble fraction are examined. The ratio in liver of group C animals was apparently (but not statistically) greater than in liver of group B rats; in contrast, the ratios in spleen, kidney and testis were approximately 40% less in group C animals. Thus, $PtCl_4$ (at 28 μmoles/kg) apparently did not inhibit the stimulated synthesis of DNA of liver in CCl_4-treated rats but did inhibit thymidine incoporation into DNA of the other three tissues, similar to the pattern seen above (Table 1).

In these experiments, the radioactivity in the acid-soluble fraction has been used as a reference for the total availability of radioactive precursor in individual tissue samples. It was of interest, therefore, to examine the distribution of radioactivity in the acid-soluble fraction. The acid-soluble fractions of liver and spleen were examined in control animals and in rats treated with 56 μmoles $PtCl_4$/kg -- the highest dose used in the prior incorporation studies.

Charcoal adsorption was used to separate intact pyrimidine compounds from their open-ring metabolites. Only those compounds which had the pyrimidine ring intact were adsorbed by charcoal from an acidic solution. At the end of the 2-hour incorporation interval, the majority of the radioactivity in the acid-soluble fraction was in the form of the open-chain metabolites and other non-adsorbed metabolites in liver and spleen of control rats (Table 5). In liver, 30% of the radioactivity in the acid-soluble fraction was in the form of compounds having the pyrimidine ring intact. Furthermore, the administration of the highest dose of $PtCl_4$ used in these experiments did not alter this distribution. In spleen the situation was similar. Approximately 22% of the radioactivity in the acid-soluble fraction of spleen of control rats was in the form

of intact pyrimidine compounds and the administration of $PtCl_4$ did not alter this value.

The charcoal-adsorbable compounds were separated by thin layer chromatography on polyethyleneimine-cellulose. Two classes of compounds were separated: (a) the thymidine phosphates, and (b) a mixture of thymidine and thymine. In liver and spleen of control animals, one-half of the total charcoal-adsorbable radioactivity was in the form of thymidine phosphates (Table 5). Administration of $PtCl_4$ did not appreciably alter the values in these two tissues.

DISCUSSION

The present study indicates that $PtCl_4$ and $Pd(NO_3)_2$ inhibit the synthesis of DNA as measured by the incorporation of radioactive thymidine. Waters et al. (6) report that the incorporation of thymidine into DNA is more sensitive to inhibition by $PtCl_4$ than the incorporation of uridine into RNA or of leucine into protein in cultured cells. The inhibition by $PtCl_4$ may be analogous to the effect of Pt-containing antitumor compounds (7-9). The inhibition of thymidine incorporation into DNA is consistent with an inhibition of DNA polymerase due to the interaction of the metallic cations with the template DNA. The interaction in vitro of the Pt-containing antitumor compounds and of Pd^{2+} ions with DNA have been demonstrated (10-12).

The structural features of the active antitumor, Pt-containing compounds have a major role in the activities of these compounds (8). It is unresolved what modifications in biological effects are made by the selection of the salt $PtCl_4$ for these studies. It is unknown whether the rates of ionization or hydration, and resultant biological effects, may be significantly different if an alternate soluble Pt^{4+} salt such as $Pt(SO_4)_2$ had been selected for these studies.

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SECTION I: Table 1.

Effect of $PtCl_4$ on thymidine incorporation into DNA of rat tissues.

		Thymidine incorporation			
		Dose of $PtCl_4$ (μmoles/kg body weight)			
		0	14	28	56
Tissue	Sample	cpm/μmole, or ratio	—% of control ± S.E. —		
Spleen	No. of samples	6	4	4	6
	DNA	1560 ±160	67* ±6	48** ±2	42** ±3
	Acid-soluble fraction	4950 ±140	105 ±4	110† ±4	105 ±5
	Ratio	0.319 ±0.039	63* ±7	43** ±2	40** ±5
Liver	No. of samples	14	4	10	8
	DNA	824 ±106	100 ±24	87 ±15	60* ±11
	Acid-soluble fraction	2050 ±180	118 ±19	113 ±10	147* ±17
	Ratio	0.428 ±0.064	100 ±47	78 ±14	45 ±14
Kidney	No. of samples	8	4	4	8
	DNA	382 ±58	99 ±19	58* ±5	50** ±4
	Acid-soluble fraction	5110 ±170	99 ±10	118† ±8	151** ±15
	Ratio	0.075 ±0.011	107 ±31	50* ±7	35** ±4
Testis	No. of samples	8	4	4	8
	DNA	402 ±74	127 ±8	75 ±10	74 ±9
	Acid-soluble fraction	6880 ±320	113† ±4	114* ±3	118** ±3
	Ratio	0.057 ±0.009	116 ±11	67 ±9	64† ±7

Table 1 (continued)

Statistical analysis (t-test): **, $P < 0.01$; *, $P < 0.05$; †, $0.05 < P < 0.10$. $PtCl_4$ and ^3H-thymidine were injected intraperitoneally at 4 hr and 2 hr, respectively, before removal of the tissues.

SECTION I: Table 2.

Effect of $Pd(NO_3)_2$ on thymidine incorporation into DNA of rat tissues.

		Thymidine incorporation			
		Dose of $Pd(NO_3)_2$ (μmoles/kg body weight)			
		0	14	28	56
Tissue	Sample	cpm/μmole, or ratio	---(% of controls ± S.E.) ----		
	No. of samples	6	4	6	7
Spleen	DNA	1550 ±200	78 ±12	52* ±12	44** ±11
	Acid-soluble fraction	5030 ±300	119† ±6	111 ±7	107 ±4
	Ratio	0.316 ±0.052	65 ±11	49* ±13	41* ±11
Liver	DNA	700 ±129	73 ±16	70 ±19	59 ±15
	Acid-soluble fraction	2230 ±520	115 ±27	138 ±22	105 ±19
	Ratio	0.342 ±0.047	60* ±6	55† ±21	55* ±10
Kidney	DNA	263 ±57	88 ±14	93 ±31	59 ±14
	Acid-soluble fraction	5590 ±100	108 ±7	104 ±3	105 ±6
	Ratio	0.048 ±0.011	84 ±20	88 ±29	56 ±13
Testis	DNA	380 ±53	107 ±7	59† ±17	40** ±9
	Acid-soluble fraction	6310 ±250	105 ±10	108 ±5	118* ±4
	Ratio	0.062 ±0.012	102 ±14	56 ±18	33** ±7

Statistical analysis (t-test): **, $P < 0.01$; *, $P < 0.05$: †, $0.05 < P < 0.10$.

$PtCl_4$ and ^3H-thymidine were injected intraperitoneally at 4 hr and 2 hr, respectively, before removal of the tissues.

SECTION I: Table 3.

Effect of $PtCl_4$ on thymidine incorporation into nuclei of of parenchymal (N-1) and stromal (N-2) cells of liver.

Experiment	A		B	
Dose of $PtCl_4$ (μmoles/kg)	0	28	0	56
No. of samples	4	4	4	4
DNA cpm/μmole ± S.E.				
Total cellular	810 ±220	910 (112%)[a] ±260	660 ±230	410 (62%)[a] ±70
N-1 nuclei	1590 ±380	1670 (105%) ±190	730 ±240	400 (55%) ±160
N-2 nuclei	700 ±140	710 (101%) ±130	450 ±60	300+ (65%) ±40
Ratio:DNA/ Acid-soluble fraction ± S.E.				
Total cellular	0.51 ±0.15	0.45 (89%) ±0.12	0.28 ±0.04	0.11* (41%) ±0.01
N-1 nuclei	0.99 ±0.28	0.83 (84%) ±0.14	0.31 ±0.05	0.11** (36%) ±0.02
N-2 nuclei	0.43 ±0.09	0.34 (79%) ±0.06	0.21 ±0.04	0.09* (41%) ±0.02
DNA N-2 nuclei/N-1 nuclei of same sample ± S.E.	0.51 ±0.15	0.42 ±0.05	0.71 ±0.10	0.82 ±0.18

[a] Expressed as % of the control values in the same experiment.

Statistical analysis (t-test): **, $P < 0.01$; *, $P < 0.05$; +, $0.05 < P < 0.10$. $PtCl_4$ and ^3H-thymidine were injected intraperitoneally at 4 hr and 2 hr, respectively, before removal of tissues.

SECTION I: Table 4.

Effect of $PtCl_4$ on thymidine incorporation into tissues of CCl_4-treated rats.

Group	A	B	C	A	B	C	A	B	C
Dose of CCl_4 (ml/kg)	0	1.0	1.0	0	1.0	1.0	0	1.0	1.0
Dose of $PtCl_4$ (μmoles/kg)	0	14	28	0	14	28	0	14	28
No. of samples [a]	4	5	4	4	5	4	4	5	4
Tissue	DNA cpm/μmole nucleotide ±S.E. (% of group B)			Acid-soluble fraction cpm/μmole nucleotide ±S.E. (% group B)			Ratio: DNA/Acid-soluble fraction ±S.E. (% of group B)		
Spleen	1800 ±200 (91)	1990 ±530	1410 ±210 (71)	3790 ±1120 (102)	3720 ±850	4420 ±490 (119)	0.57 ±0.16 (82)	0.70 ±0.37	0.35 ±0.11 (50)
Liver	580 ±130 (10)	6070 ±1540	7610 ±1940 (125)	1850 ±90 (58)	3180 ±690	2710 ±1030 (85)	0.32 ±0.09 (16)	2.00 ±0.50	3.15 ±0.46 (157)
Kidney	300 ±60 (100)	300 ±50	200 ±40 (67)	6240 ±1150 (108)	5790 ±290	7090 ±1530 (122)	0.052 ±0.012 (100)	0.052 ±0.009	0.032 ±0.009 (61)
Testis	540 ±80 (114)	470 ±50	310* ±40 (65)	7450 ±120 (106)	7050 ±290	6860 ±360 (97)	0.073 ±0.012 (108)	0.067 ±0.005	0.039+ ±0.011 (58)

Statistical analysis (t-test): *, $P < 0.05$; +, $0.05 < P < 0.10$.

CCl_4, diluted in corn oil, was injected intraperitoneally at 42 hr, $PtCl_4$ at 4 hr, and ^{3}H-thymidine at 2 hr before removal of the tissues.

[a] Except 3 rats each in spleen samples of groups A and B.

SECTION I: Table 5.

Distribution of ^{3}H-labeled compounds in the acid-soluble fraction.

Tissue	Liver		Spleen	
Dose of $PtCl_4$, µmoles/kg	0	56	0	56
Charcoal adsorption, % of ^{3}H in acid-soluble fraction				
intact pyrimidine compounds (adsorbed)	30 (28; 31)	28 (22; 34)	22 (20; 24)	23 (19; 26)
pyrimidine catabolites (not adsorbed)	70 (69; 72)	72 (66; 78)	78 (76; 80)	77 (74; 81)
Thin layer chromatography, % of ^{3}H of charcoal-adsorbable fraction				
thymidine phosphates	48 (47; 48)	44 (37; 51)	54 (54; 55)	59 (54; 64)
thymidine and thymine mixture	43 (43; 44)	47 (42; 52)	41 (41; 42)	36 (33; 40)

Means of values of two rats; percentage values of each rat are given in parentheses.

$PtCl_4$ and ^{3}H-labeled thymidine were injected intraperitoneally 4 hr and 2 hr, respectively, before removal of the tissues.

SECTION II. EFFECTS OF PLATINUM AND PALLADIUM SALTS ON PARAMETERS OF DRUG METABOLISM IN THE RAT

INTRODUCTION

Platinum and palladium compounds are used as the active components in the catalytic converters of air pollution control devices of various motor vehicles. It is of interest, therefore, to determine the biological effects of the salts of these metals.

The acute exposure of rats to various metallic cations such as Cd^{2+} and Pb^{2+} markedly decreases the parameters of the hepatic microsomal mixed-function oxidases.[1-3] However, longer-term administration of these metallic salts to rats typically does not result in impaired microsomal enzymatic activities.[4-6] Likewise, in the current study, the injection of Pt or Pd salts (or in some cases, 1-week dietary administration) results in decreased activities of hepatic microsomal "drug metabolizing" enzymes whereas dietary administration of 4 weeks or longer did not decrease these activities.

MATERIALS AND METHODS

Materials. Male Sprague-Dawley rats, obtained from Zivic-Miller Laboratories, were used in all experiments. The Pt and Pd salts were purchased from the following sources: B.F. Goldsmith Chemical and Metal, $Pt(SO_4)_2 \cdot 4H_2O$, PtO_2, $PtCl_4$, PdO, $PdCl_2$, $PdSO_4$; Research Organic/Inorganic Chemical, $PtCl_2$, $PdCl_2.2H_2O$, $PtCl_4$, $Pd(NO_3)_2$ aqueous solution, PtO_2; Var-Lac-Oid Chemical, $Pt(SO_4)_2.4H_2O$; Ventron/Alfa Products, PdO, $PtCl_4$; ICN/K and K Laboratories, $Pt(SO_4)_2.4H_2O$, $PdSO_4$; and Apache Chemicals, $PtCl_2$. Glucose-6-phosphate, NADP, and glucose-6-phosphate dehydrogenase (type XV) were obtained from Sigma Chemical.

Diet treatments. The dietary administration of metallic salts was conducted for one week (7.5-8.5 days), four weeks (28.5-32.5 days), or 13 weeks (87-93 days). The rats were housed four rats/cage. When initially placed on metal-containing diets, the mean body weights were 100-110 g (age, 4-5 weeks). Body weights of individual rats and consumption of feed and fluid (per cage) were measured every seventh day. The metallic salts were administered either in the drinking fluid or by mixing in the dry feed (Purina laboratory chow). The volume of fluid consumed (in ml) was approximately 1.6 times the weight of feed consumed (in grams).

Isolation of microsomes and assay methods. Rats were fasted for 14 hr before isolation of microsomes. Liver was homogenized in 0.15 M KCl-50 mM Tris-HCl (pH 7.7 at 5°). The homogenate was centrifuged at 9000 g for 20 min; the resulting supernatant was then centrifuged at 159,000 g-av. for 30 min. The microsomal pellet was resuspended in 0.15 M KCl-50 mM Tris-HCl and recentrifuged at 159,000 g-av. for 30 min. The washed microsomes were resuspended in 0.1 M Tris-HCl for transfer to incubation mixtures (final pH, 7.6-7.7 at 37°). Glucose-6-phosphate, glucose-6-phosphate dehydrogenase

and NADP were used as the NADPH-generating system. Aniline hydroxylase was measured (at 37° and 1.5-2.0 mg microsomal protein/ml) by the method of Imai et al.,[7] modified by the addition of $HgCl_2$[8] during the assay for p-aminophenol. Aminopyrine demethylase was measured (at 37° and 1.5-2.0 mg microsomal protein/ml) by the formation of formaldehyde (Nash reaction).[9] Protein was measured by the method of Lowry et al.[10] Microsomal cytochrome P-450 and cytochrome b_5 were analyzed essentially by the methods of Omura and Sato.[11]

The isolated hepatic microsomes were routinely assayed for the following parameters of drug metabolism: yield of microsomal protein (mg/g liver), aniline hydroxylase activity (nmoles p-aminophenol produced/min/mg microsomal protein and nmoles p-aminophenol produced/min/nmole cytochrome P-450), aminopyrine demethylase activity (nmoles formaldehyde produced/min/mg microsomal protein and nmoles formaldehyde produced/min/nmole cytochrome P-450), and microsomal content of cytochrome P-450 and of cytochrome b_5 (each expressed as nmoles/mg microsomal protein). Aminopyrine and aniline were selected as representatives of substrates which give type I and type II binding spectra, respectively. The data in the tables are expressed as the percentage of the mean control values and the control values are given the legends.

Effects of addition of metallic salts to the incubation medium. In all cases the incubation medium contained 5 mM $MgCl_2$. The addition of $PtCl_4$, $Pd(NO_3)_2$ and $PbCl_2$ to the incubation medium at the concentrations used caused negligible changes (< 0.1) in the pH of the medium; the addition of $MnCl_2 \cdot 4H_2O$ did cause some decrease in the pH of the medium. The addition of the salts to the incubation medium did not result in the formation of a microsomal sediment or in an observable turbidity. The addition of the metallic salts in vitro, at the concentrations used, did not inhibit the

activity of the NADPH-generating system (glucose-6-phosphate, glucose-6-phosphate dehydrogenase and NADP).

RESULTS

Hexobarbital-induced sleeping time. The effects of intraperiotoneal injections of $PtCl_4$ or $Pd(NO_3)_2$ on the hexobarbital-induced sleeping time in rats are presented in Table 1. The metallic salts were administered for two consecutive days, each day at the designated dose, and sleeping times were measured on the third day. $PtCl_4$, at doses of 28 μmoles (5.5 mg Pt^{4+}) and 56 μmoles (10.9 mg Pt^{4+})/kg body weight, increased hexobarbital-induced sleeping times by approximately 25 and 50%, respectively. The higher dose of $PtCl_4$ is equal to one-half of the intraperitoneal LD_{50} dose of 113 μmoles/kg body weight (administered as a single dose with a 14-day observation interval) but is less than the intraperitoneal LD_5. The two-day intraperitoneal administration of $Pd(NO_3)_2$ at 56 μmoles (6.0 mg Pd^{2+}) or 113 μmoles (12 mg Pd^{2+})/kg body weight also increased the hexobarbital-induced sleeping time by approximately 60% (Table 1). Thus, the administration of $PtCl_4$ or $Pd(NO_3)_2$ apparently decreased the ability of the treated animals to metabolize hexobarbital in vivo.

Acute effects on microsomal enzymatic activities. Various parameters of drug metabolism were measured in hepatic microsomes isolated from rats injected with $PtCl_4$ or $Pd(NO_3)_2$ at 18 and 42 hr before isolation of the microsomes. The intraperitoneal injection of $PtCl_4$ for two consecutive days prior to the isolation of the microsomes generally produced only small changes in the measured parameters of drug metabolism (Table 2). Decreases of approximately 15-25% were observed in the microsomal content of cytochrome P-450 (nmoles/mg microsomal protein) and in the aminopyrine demethylase activity (per mg microsomal protein), respectively. Small charges (<15%) also were observed in the microsomal content of cytochrome b_5 (nmoles/mg microsomal protein) and in the yield of hepatic microsomal protein (mg protein/g liver). Thus, at doses relatively high in comparison to the LD_{50}, injection

of $PtCl_4$ had only moderate effect on the measured parameters of drug metabolism. Injection of $Pd(NO_3)_2$ decreased the aminopyrine demethylase activity (per mg microsomal protein) by one-third at doses of 56 or 113 μmoles/kg body weight (Table 2). The microsomal content of cytochromes P-450 and b_5 were also decreased but by only one-fifth and about one-fourth, respectively.

In an additional series of experiments (data not shown), each Pt-treated rat received a single intraperitoneal injection of $PtCl_4$ (100 μmoles (19.5 mg Pt)/kg body weight) and hepatic microsomes were isolated 45 hr later. The following parameters of drug metabolism were reduced in microsomes of the Pt-treated rats: the yield of microsomal protein (mg/g liver), by 24% ($P<0.001$); aniline hydroxylase (nmoles p-aminophenol produced/min/mg protein), by 28% ($P<0.01$); aminopyrine demethylase (nmoles formaldehyde produced/min/mg protein), by 42% ($P<0.01$); and microsomal content of cytochrome P-450 (nmoles/mg microsomal protein), by 33% ($P<0.01$). The microsomal content of cytochrome b_5 was reduced by only 13% ($P>0.1$).

Effect of dietary administration on microsomal activities. One of the objectives in this study was to determine the effects of " long-term, low-level" (dietary) administration of the metallic salts on the ability of isolated microsomes to function in drug metabolism. With one exception, the dietary administration (via drinking fluid or solid feed) of Pt^{4+} or Pd^{2+} salts resulted in the following pattern of changes (Table 3). (a) If a 1-week metal-containing diet resulted in any changes, there was a decrease in the parameters of drug metabolism (e.g., activities of aniline hydroxylase and aminopyrine demethylase), consistent with the acute effects of the intraperitoneally injected metallic salts. (b) If a 4- or 13-week metal-containing diet resulted in any changes, there was an increase in the parameters of drug metabolism

The dietary administration of $PtCl_4$ had relative little effect on any of the measured parameters of drug metabolism (Table 3). The only changes which appear to occur were increases of 20-30% in aminopyrine demethylase, aniline hydroxylase and/or cytochrome b_5 after dietary administration of $PtCl_4$ for 4 weeks at 13.2 mmoles (2.58 g Pt^{4+})/kg solid feed or for 13 weeks at 0.54 mmoles (106 mg Pt^{4+})/liter drinking fluid; each rat consumed a mean of 1.58 g and 1.4 g, respectively, of Pt^{4+} during the diet treatments. A one-week dietary treatment with $Pt(SO_4)_2 . 4H_2O$ showed a decreased activity of aniline hydroxylase whereas a four-week treatment showed little or no change in the enzymatic activities. PtO_2, an insoluble salt, had marginal effects on the measured parameters even when the concentration in the feed was 29.8 mmoles (5.81 g Pt^{4+})/kg feed; each rat consumed a mean of 4.9 g of Pt^{4+} during the four weeks on the diet.

In each of two experiments, the administration of $PdCl_2 . 2H_2O$ (a partially soluble salt) as a saturated solution in the drinking fluid for one week, decreased the activities of aniline hydroxylase and aminopyrine demethylase in isolated microsomes (Table 3). In contrast, the 4-week dietary administration

of $PdCl_2$ (an "insoluble" salt), at 13.2 mmoles (1.40 g Pd^{2+})/kg feed, resulted in an increase in aniline hydroxylase and aminopyrine demethylase (per mg microsomal protein) with an equivalent increase in the microsomal content of cytochrome P-450. The increase in enzymatic activities did not occur when the dietary concentration of $PdCl_2$ was increased to 29.8 mmoles (3.17 g Pd^{2+})/kg feed but the Pd-treated rats in the latter experiment showed a 25% reduction in weight gain.

The dietary administration of $PdSO_4$ did not produce any (statistically significant) changes in the parameters of drug metabolism even when administered at a concentration of 29.8 mmoles (3.17 Pd^{2+})/kg feed. Likewise, PdO caused no changes in the measured parameters except for a decrease in the yield of microsomal protein at a PdO level of 29.8 mmoles (3.17 g Pd^{2+})/kg feed.

Dietary $PbCl_2$, 4 weeks at 29.8 mmoles (6.17 g Pb^{2+})/kg feed, increased the yield of microsomal protein and decreased the activity of aniline hydroxylase; other diet schedules produced only minor changes (Table 3). $MnCl_2.4H_2O$, administered in the drinking fluid for 13 weeks at 8.3 mmoles (0.46 g Mn^{2+})/liter or 18.6 mmoles (1.02 g Mn^{2+})/liter, did not alter any of the measured parameters of drug metabolism.

Effect of prior administration of Pt^{4+} on survival following an LD_{50} dose of $PtCl_4$. Decreases in the ability of isolated microsomes to metabolize drugs in vitro are observed only following the acute administration (i.e., 18 and 42 hr prior to isolation of microsomes) of $PtCl_4$ or $Pd(NO_3)_2$ or following a short diet period (i.e., one week). After 4- or 12-week diet periods, the drug-metabolizing activities of isolated microsomes from Pt^{4+}-treated (or Pd^{2+}-treated) rats generally were equal to or greater than those from controls rats. Such a pattern has been observed for Cd^{2+} and Pb^{2+} salts. In the case of Cd^{2+}, the toxicity is reduced by the induced synthesis of a Cd^{2+}-binding protein in rats treated with Cd^{2+} or Zn^{2+} salts. In such cases, the prior administration of Cd^{2+} (or Zn^{2+}) protects rats against a subsequent

administration of a normally lethal dose of Cd^{2+} salts.[12,13] A similar type of study was conducted with $PtCl_4$. In rats weighing approximately 270 g, one group of rats received an intraperitoneal injection of $PtCl_4$ (56 μmoles/kg body weight) and control rats received saline injections. After 48 hr, each rat received an intraperitoneal injection of $PtCl_4$ (113 μmoles/kg). After one week following the higher dose of $PtCl_4$, 33% and 100% of the saline-pretreated and of the Pt^{4+}-pretreated rats, respectively, survived; there were a total of 12 rats in each of the groups. Although an induced synthesis of a Pt^{4+}-binding protein in the Pt^{4+}-pretreated rats has not been demonstrated, the observed protection against $PtCl_4$-lethality by Pt^{4+}-pretreatment is consistent with the production of such a protein and might be analogous to findings with Cd^{2+} where the production of such a protein has been experimentally demonstrated.[14]

Effect of addition of cations to the incubation medium on activity of aminopyrine demethylase. The alterations in the activity of aminopyrine demethylase of isolated hepatic microsomes from control rats were measured after the addition of various metallic cations to the incubation medium. The Pt^{4+} and Pd^{2+} ions were appreciably more inhibitory than Pb^{2+} or Mn^{2+} ions. At aminopyrine concentrations of 0.25 or 1 mM, 50% inhibition was obtained at 0.15-0.2 mM Pt^{4+} (Fig. 1A).

However, the extent of inhibition of aminopyrine demethylase by Pt^{4+} is markedly decreased as the concentration of the substrate (aminopyrine) is increased. At an aminopyrine concentration of 2 mM, 50% inhibition of the demethylase occurred at 0.5 mM $PtCl_4$. At aminopyrine concentrations of 1 or 4 mM, 50% inhibition occurred at 0.2-0.3 mM Pd^{2+} (Fig. 1B). In contrast to the pattern with Pt^{4+}, however, the inhibition in vitro of aminopyrine demethylase by Pd^{2+} is not affected by changes in the aminopyrine concentration. A 4-fold increase in aminopyrine concentration (i.e., 1 and 4 mM) altered the percentage inhibition by Pd^{2+} by <5%.

Pb^{2+}, at concentrations of 0.1-1.0 mM (and at 1-4 mM aminopyrine), produced only 15-25% inhibition of the aminopyrine demethylase activity of isolated microsomes. Mn^{2+}, at either 1 or 4 mM aminopyrine, inhibited the aminopyrine demethylase approximately 20% and 40% at 6.4 and 12.8 mM Mn^{2+}, respectively. However, a $MnCl_2$-induced change in the pH of the incubation medium may contribute to the lower activity seen at the higher Mn^{2+} concentration.

The kinetics of the aminopyrine demethylase reaction in the presence of Pt^{4+} or Pd^{2+} are shown in Fig. 2. The differing response to inhibition of aminopyrine demethylase by Pt^{4+} and Pd^{2+} to increases in aminopyrine concentration resulted in a different character in the inhibition. Double reciprocal plots (i.e., 1/v versus 1/S) in the Pt^{4+}-inhibited demethylase reaction gave a common intercept on the vertical axis (i.e., competitive inhibition) (Fig. 2A) whereas plots in the Pd^{2+} -inhibited reaction had a common intercept at, or very near, the horizontal axis (i.e., non-competitive inhibition) (Fig. 2B). However, the patterns of the Lineweaver-Burk plots shown in Fig. 2A and 2B occurred only where the concentrations of Pt^{4+} and Pd^{2+} were relatively low (e.g., 0.1 mM $PtCl_4$ and 0.2 mM $Pd(NO_3)_2$). Higher concentrations of either metal (e.g., 0.20-0.25 mM Pt^{4+} or 0.4 mM Pd^{2+}) caused an inhibition in which the plots of 1/v versus 1/S of control and metal-containing samples generally did not have common intercepts on either axis.

DISCUSSION

In the acute studies (Table 1), the $PtCl_4$ and $Pd(NO_3)_2$ were injected intraperitoneally each day for two consecutive days before isolation of the microsomes. With this schedule, decreases were observed in the aminopyrine demethylase activity (per mg microsomal protein) and the microsomal content of cytochrome P-450. However, the administration of methylmercury,[1,2] $PbCl_2$,[2] or cadmium acetate,[3] at (generally) appreciably lower molar doses, caused appreciably greater decreases in the microsomal content of cytochrome P-450 and in the enzymatic activities which utilize cytochrome P-450 when measured 1-2 days after single or 2-consecutive-day administration. Thus, on a molar basis and at short intervals after administration, the Pt^{4+} and Pd^{2+} salts produced appreciable lesser effects on the parameters of drug metabolism than the effects reported for the salts of methylmercury, Pb^{2+} or Cd^{2+}.

The decreases in the parameters of microsomal drug metabolism in Pt^{4+}- or Pd^{2+}-treated rats were observed only after short-term exposure to the metals (i.e., after intraperitoneal injection or 1-week diets). After longer exposure (4- or 12-week diets), Pt^{4+} or Pd^{2+} salts produced no effect or small increases in the parameters of microsomal drug metabolism. Thus, the Pt^{4+} and Pd^{2+} salts produce a pattern of effects similar to the pattern reported for Cd^{2+} and Pb^{2+}. Although Cd^{2+} salts[3] and Pb^{2+} salts[2] produce marked decreases in parameters of drug metabolism at short intervals after administration, the long-term dietary administration of Cd^{2+} salts[4] or Pb^{2+} salts[5,6] do not alter or may increase various parameters o drug metabolism.

Depending on the aminopyrine concentration, 25% and 50% inhibition of aminopyrine demethylase activity can be obtained in vitro at 0.1 and at approximately 0.2 mM $PtCl_4$, respectively. One of the lower dietary levels of Pt used in the present experiments was 1.63 mmoles (318 mg Pt^{4+})/liter

of drinking fluid. Rats on this diet consumed 60-80 mg Pt in an 8-9 day interval and attained maximum Pt levels of 4.8 μg Pt/g kidney (nominal concentration of 35 μM, assuming tissue water of 70%) and 0.8-2.2 μg Pt/g liver (nominal concentration of 6-16 μM).[15] Rats which survived for 14-days after receiving an intraperitoneal dose of $Pt(SO_4)_2.4H_2O$ (113 mg Pt/kg body weight) (equivalent to 90% of the LD_{50}) attained liver and kidney levels of approximately 35 μg Pt/g tissue (a nominal concentration of 0.25 mM).[15] Thus, although levels of Pt in tissues of experimental animals can attain levels which are nominally sufficient to inhibit the representative drug-metabolizing enzyme studied here, the amounts administered to the animals far exceed an anticipated environmental exposure.

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SECTION II. Table 1.

Increase in hexobarbital-induced sleeping times in rats treated with $PtCl_4$ or $Pd(NO_3)_2$.

Dose of metallic salt (μmoles/kg body weight)	Hexobarbital-induced sleeping time (% of mean, paired controls ± S.E.)	
	$PtCl_4$	$Pd(NO_3)_2$
0	100 ± 16	100 ± 8
14	111 ± 10	--
28	123 ± 10	--
56	151 ± 15*	159 ± 20*
113	--	160 ± 23*

Rats, initially weighing 162 g (± 13, S.D.) were injected intraperitoneally with $PtCl_4$ or $Pd(NO_3)_2$ 42 and 18 hr (each time at the designated dose) prior to the intraperitoneal injection of hexobarbital (100 mg/kg body weight). The mean hexobarbital-induced sleeping times of control rats were 43 min in both the $PtCl_4$ and $Pd(NO_3)_2$ experiments. There were 6-7 and 8-11 values for each dose in the $PtCl_4$ and $Pd(NO_3)_2$ experiments, respectively. Statistical analysis (t-test): *, $P < 0.05$.

SECTION II. Table 2.

Effect of intraperitoneal injections of $PtCl_4$ or $Pd(NO_3)_2$ for two consecutive days on parameters of drug metabolism by isolated hepatic microsomes (MCS).

Treatment	Dose (μmoles salt/kg body wt.)	Number values	Microsomal protein yield (mg/g liver)	Aminopyrine demethylase (per mg microsomal protein	Aminopyrine demethylase (per nmoles cytochrome P-450)	Cytochrome P-450 (nmoles/ mg MCS protein)	Cytochrome b5 (nmoles/ mg MCS protein)
			(% of mean paired controls ± S.E.)				
Control	-	10	100 ±2	100 ±3	100 ±8	100 ±7	100 ±4
$PtCl_4$	3.5	3	-	92 ±9	88 ±6	104 ±13	101 ±4
	14.1	6	91+ ±2	83* ±5	93 ±8	86+ ±4	97 ±2
	28.2	5	94 ±3	83** ±2	99 ±7	81* ±5	89+ ±2
	56	4	91+ ±4	85 ±13	115 ±16	74** ±3	87* ±3
Control	-	7	100 ±2	100 ±4	100 ±11	100 ±7	100 ±6
$Pd(NO_3)_2$	28	3	100 ±4	103 ±15	92 ±2	112 ±18	111 ±11
	56	5	100 ±4	69** ±6	84 ±7	81 ±10	86 ±5
	113	4	96 ±5	62* ±14	74+ ±5	80 ±15	72* ±7

Rats, initially weighing 163 g (± 12, S.D.) were injected intraperitoneally with $PtCl_4$ or $Pd(NO_3)_2$ 42 and 18 hr (each time at the designated dose) prior to isolation of hepatic microsomes. Values (mean ± S.D.) for 14 control rats were: initial body

Table 2 (Continued)

weight, 161 ± 11 g; liver weight, 5.5 ± 0.7 g; yield of microsomal protein, 25 ± 2 mg/g liver; aminopyrine demethylase, 5.9 ± 0.9 nmoles formaldehyde produced/min/mg protein; microsomal cytochrome P-450 content, 0.58 ± 0.16 nmoles/mg protein; and microsomal cytochrome b5 content, 0.29 ± 0.05 nmoles/mg protein. Statistical analysis: **, $P < 0.01$; *, $P < 0.05$; +, $0.05 < P < 0.10$; no designation used where $P > 0.10$.

SECTION II. Table 3.

Effects of administration of metal-containing diets on the parameters of drug metabolism by isolated hepatic microsomes (MCS).

Metallic salt	Diet duration (weeks)	Dietary metal concn. (nmoles/ l or kg)	Number rats[a]	Microsomal protein yield (mg/g liver)	Aniline hydroxylase		Aminopyrine demethylase		Cytochrome P-450 (nmoles/ mg MCS protein)	Cytochrome b_5 (nmoles/ mg MCS protein)
					(activity/ mg MCS protein)	(activity/ nmoles cytochrome P-450)	(activity/ mg MCS protein)	(activity/ nmoles cytochrome P-450)		
				(% of paired mean controls)						
$PtCl_4$	1	1.63/1	12	101	100	-	106	-	nm	nm
	1	2.45/1	4	84*	110	-	102	-	-	115
	4	1.63/1	12	98	97	-	100	-	-	97
	4	5.9/kg	4	101	88	-	99	-	-	94
	4	13.2/kg	4	97	92	-	122+	117	102	129*
	13	0.54/1	4	95	123+	128*	114	120	97	107
$Pt(SO_4)_2 \cdot 4H_2O$	1	1.63/1	8	103	79*	-	-	-	nm	nm
	4	5.9/kg	4	110+	88	-	102	-	nm	nm
PtO_2	4	29.8/kg	4	103	104	109	110	120	93	94
$PdCl_2 \cdot 2H_2O$	1	(satd.soln)	8	102	72**	-	78**	-	nm	nm
$PdCl_2$	4	13.2/kg	8	95	121**	98	125**	101	125**	110*
	4	29.8/kg	4	104	96	87	100	92	109	103

Table 3. Continued

Metallic salt	Diet duration (weeks)	Dietary metal concn. (μmoles/l or kg)	Number rats[a]	Microsomal protein yield (mg/g liver)	Aniline hydroxylase (activity/mg MCS protein)	Aniline hydroxylase (activity/nmoles cytochrome P-450)	Aminopyrine demethylase (activity/mg MCS protein)	Aminopyrine demethylase (activity/nmoles cytochrome P-450)	Cytochrome P-450 (nmoles/mg MCS protein)	Cytochrome b_5 (nmoles/mg MCS protein)
				(% of paired mean controls)						
$PdSO_4$	1	(satd. soln.)	4	101	109	102	88	81	108	99
	4	(satd. soln.)	4	98	90	98	96	106	92	92
	4	29.8/kg	4	97	93	82+	102	90	111	100
PdO	4	29.8/kg	4	84*	97	96	93	89	106	99
$PbCl_2$	4	3.7/1	8	104	92	-	91	-	-	93
	4	8.3/1	4	103	101	112	nm	-	90	99
	4	29.8/kg	8	110*	89+	79*	97	-	110	107*
	13	3.7/1	6	94	86	-	-	-	nm	nm
$MnCl_2 \cdot 4H_2O$	13	8.3/1	6	102	92	-	95	-	nm	nm
	13	18.6/1	4	99	107	112	102	108	95	102

[a]An equal number of rats were used for control animals.

Each experiment was conducted generally with 4 control and 4 metal-treated rats and several experiments were repeated; nm, not measured. Values (mean ± S.D.) for 92 control rats (except 68 rats for cytochromes P-450 and b_5) in the 4-week diet

experiments were: liver weight, 10.5 ± 1.5g; yield of microsomal protein, 26 ± 3 mg/g liver; aminopyrine demethylase, 7.4 ± 1.4 nmoles formaldehyde produced/min/ mg protein; aniline hydroxylase, 1.0 ± 0.2 nmoles p-aminophenol produced/min/mg protein; microsomal cytochrome P-450 content, 0.70 ± 0.17 nmoles/mg microsomal protein; and microsomal cytochrome b_5 content, 0.32 ± 0.04 nmoles/mg microsomal protein. Statistical analysis (t-test): **, $P < 0.01$; *, $P < 0.05$; †, $0.05 < P < 0.10$.

LEGENDS TO FIGURES

Fig. 1. Effect of Pt^{4+} and Pd^{2+} addition to the incubation medium on the aminopyrine demethylase activity in vitro. $PtCl_4$ (1A) or $Pd(NO_3)_2$ (1B), the concentrations expressed on a logarithmic scale, were added to the standard incubation medium for aminopyrine demethylase. It should be noted that the medium contains 5 mM $MgCl_2$. There were 6-9 and 3-4 values for each Pt^{4+} point and Pd^{2+} point, respectively. The vertical lines show the standard error. (A) Pt^{4+}; aminopyrine at Δ, 0.25 mM; 0, 1 mM; ■, 2 mM; and □, 4 mM. (B) Pd^{2+}; aminopyrine at 0, 1 mM; and □, 4 mM.

Fig. 2. Double reciprocal plots of the inhibition of aminopyrine demethylase by the addition of $PtCl_4$ or $Pd(NO_3)_2$ to the incubation medium. The lines drawn are the calculated least-squares regression lines. In the six experiments on the kinetic parameters, the V_{max} of aminopyrine demethylase of control hepatic microsomes was 7.3 ± 0.4 (mean ± S.E.) nmoles formaldehyde/min/mg protein and the Km was 0.93 ± 0.23 mM. 1/v, 1/(nmoles formaldehyde produced/10 min/mg protein); 1/S, 1/(aminopyrine, expressed in mM). The incubation medium contains 5 mM $MgCl_2$. Each point is the mean of duplicate samples. (A) 0, control; Δ, 0.1 mM $PtCl_4$. (B) 0, control; □, 0.2 mM $Pd(NO_3)_2$.

Figure 1

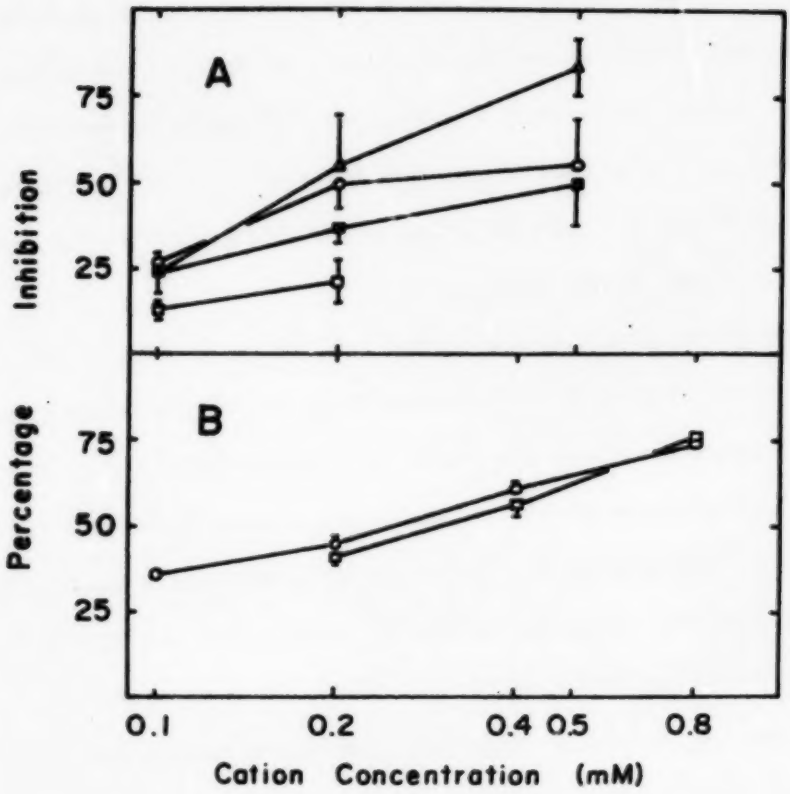
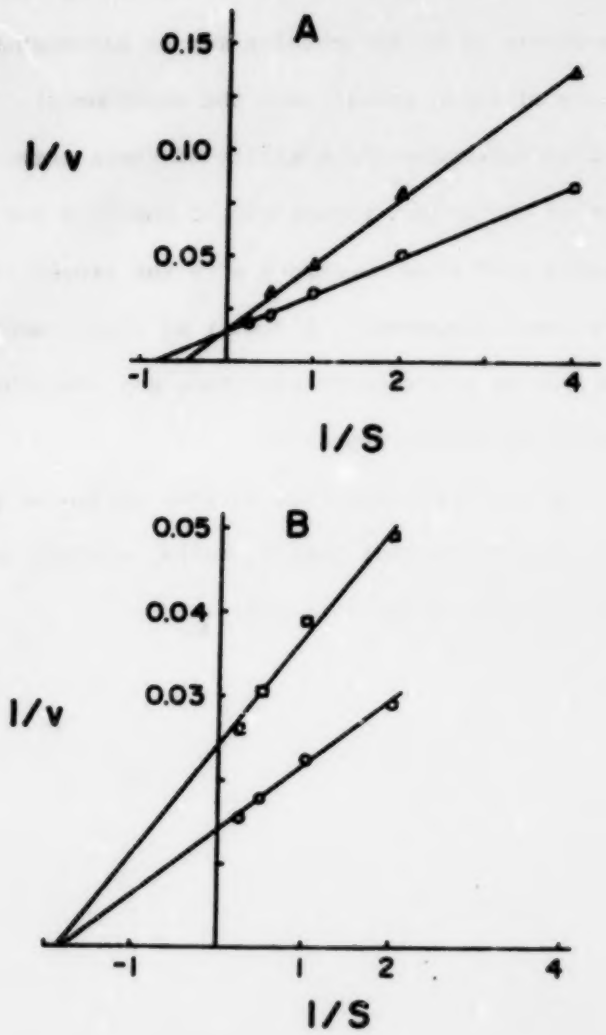

A
B
75
50
25
Inhibition
Percentage
0.1
0.2
0.4
0.5
0.8
Cation Concentration (mM)

Figure 2



SECTION III. TOXICITY OF PLATINUM AND PALLADIUM SALTS IN THE RAT

INTRODUCTION

The incorporation of platinum and palladium as components in the catalytic converters of motor vehicles may be accompanied by the release of various salts of these metals into the environment. Consequently, it is of interest to determine the toxicity of these compounds in mammalian systems. Most of the prior studies on the toxicity and biochemical actions of platinum salts have been concerned with the properties of platinum-containing antitumor compounds. A number of these compounds interact with tissue macromolecules and such interactions may contribute to the biochemical and toxic effects of these compounds.

Since it has been proposed that various manganese compounds be substituted for the tetraethyl lead in fuels, various compounds of manganese and lead are included in this study.

MATERIALS AND METHODS

All studies were conducted with male Sprague-Dawley rats obtained from Zivic-Miller Laboratories. The animals were received at 3-3.5 weeks of age and were maintained for 1-1.5 weeks before use. The mean body weights were usually 100-110 g when the rats were used for lethal-dose studies or started on the diets.

In the lethal dose experiments, a 14-day observation interval was used and survivors were weighed 7 and 14 days after treatment. The LD_{50} values were calculated by the method of Litchfield and Wilcoxon (1949). In diet experiments, four rats were maintained per cage and, at 7-day intervals, the following measurements were made: body weight of individual animals and consumption of feed and drinking fluid per cage. The metallic salt under study was either dissolved in the drinking fluid or mixed in the ground dry feed. Animals consumed feed and drinking fluid ad libitum. In control animals, the volume of fluid consumed (in ml) was approximately 1.6 times the weight of the feed consumed (in grams). Analyses for metals were performed on samples from three lots of feed (Purina Laboratory Chow); the feed contained (mean $\pm$ S.D.): 56 $\pm$ 5 mg Mn/kg feed and 0.99 $\pm$ 0.07 mg Pb/kg feed. The analyses for platinum in the three lots were 0.09, < 0.02 and < 0.02 mg/kg.

At the end of the diet intervals the rats were weighed, then fasted for 14-15 hr, and tissues removed and weighed. The rats were fasted because parameters of drug metabolism in vitro were measured on isolated hepatic microsomes (to be reported elsewhere). Tissue samples were frozen for the analyses of metals and for the measurement of the tissue content of DNA, RNA and protein. For the latter analyses, tissue samples

were homogenized in water, and tissue macromolecules were precipitated and washed with cold 0.5 M $HClO_4$. The RNA was hydrolyzed in 0.5 M NaOH (1 hr, 37°C) and the DNA and RNA were precipitated and washed with cold 0.5 M $HClO_4$. The DNA was then hydrolyzed in 0.5 M $HClO_4$ (20 min, 90°C). The DNA and the RNA were determined from the absorbance at 260 nm (Gilford model 2400 spectrophotometer). The protein in the final precipitate was measured by the method of Lowry et al. (1951).

From a least-squares linear regression line of organ weight versus body weight for all control animals, an "expected weight" was calculated. Statistical comparisons were made on the organ weights of metal-treated rats and their paired controls, each value being expressed as a percentage of the "expected weight". This method corrects for the change in organ weights (expressed as percentage of body weight) which occurs with changes in body weight. The equations of the expected wet weight of organs of control rats (fasted 14-15 hr before removal of the tissues) in the weight range of 130-620 g; i.e., after 1-, 4- and 12-week diets, were: liver weight (g) = 0.0240 (body weight, g) + 2.66; kidney weight (g) = 0.00624 (body weight, g) + 0.88; spleen weight (g) = 0.000705 (body weight, g)+ 0.80; heart weight (g) = 0.00211 (body weight, g) + 0.27, and testis weight (g) = 0.00559 (body weight, g) + 1.19. Statistical analyses of organ weights and of growth rates were made by the t-test.

RESULTS

Lethal dose studies. The LD_{50}, LD_{10} and LD_{90} of the various metal salts, after intraperitoneal or oral (stomach tube) administration, are given in Table 1. Various other salts were examined in lethal dose experiments but lack of acute toxicity at the highest oral doses tested did not permit determination of LD_{50} values. Those salts which caused lower levels of lethality after oral administration were the following (percentage survival given, with number of rats tested in parentheses): 60% (10) and 82% (11) at MnO_2 doses of 115 and 77 mmoles/kg, respectively; 90% (10) at a $PbCl_2$ dose of 35 mmoles/kg; 64% (11) at a PbO dose of 45 mmoles/kg; 100% (6) at a PdO dose of 82 mmoles/kg; 71% (7) and 83% (6) at PtO_2 doses of 35 and 20 mmoles/kg, respectively.

Thus, following the intraperitoneal administration of the metallic salts, the acute toxicities of the salts (expressed on a molar basis) were in the following order: $PtCl_4 > Pt(SO_4)_2.4H_2O = PdCl_2.2H_2O = MnCl_2.4H_2O > PdSO_4 > PtCl_2 > PbCl_2$. Likewise, following the oral administration of the metallic salts, the toxicities of the salts (expressed on a molar basis) were in the following order: $PtCl_4 > Pt(SO_4)_2.4H_2O > PdCl_2.2H_2O > RuCl_3 > MnCl_2.4H_2O > PbO = PtO_2 > MnO_2 >$ PdO. Thus, $PtCl_4$ was the most toxic salt tested by either route of administration and when expressed on either a molar basis or as weight of cation.

Weight gains. The weight gain/rat was determined for the following dietary intervals: each week during weeks 1-4 and, in the 12-week experiments, for the fifth through eighth week and for the nineth through twelveth week (expressed as g/week/rat). The inclusion of $MnCl_2.4H_2O$ in the drinking fluid at levels of 8.3 or 18.6 mmoles/liter (1.64 and 3.69 g salt/liter, respectively) did not alter the weight gain during any interval of the 12 weeks on the diet. Likewise, $PbCl_2$ in the drinking fluid at a level of 3.7 mmoles/liter (1.02 g salt/liter) did not affect weight gain during any of the intervals through the 12 weeks on the diet. At a $PbCl_2$ concentration of 8.3 mmoles/liter (2.30 g $PbCl_2$/liter) in the drinking fluid, the weight gain/week/rat of the Pb-treated rats did not differ from controls each week on a 4-week schedule ($P > 0.1$) but the total weight gain during the entire 4 weeks was 79% ($P > 0.1$) of that of controls. Increasing the dietary intake by administration of $PbCl_2$ in the feed at the levels 13.2 or 29.8 mmoles/kg feed (1.4 or 3.3 g salt/kg feed, respectively) resulted in decreased weight gain during the first week at the lower level and for each of the first two weeks at the higher level (Table 2). However, for the last 2-3 weeks of the 4-week diets, weight gains by the $PbCl_2$-treated rats were equal to that by controls (Table 2).

The use of a saturated solution of $PdCl_2.2H_2O$ (a "partially soluble" salt) as the drinking fluid for one week did not alter the weight gain of the Pd-treated rats. Likewise, the addition of $PdCl_2$ (an insoluble salt) to feed at a level of 13.2 mmoles (2.34 g salt)/kg feed did not decrease the weight gain by Pd-treated rats during each of the 4 weeks on the diet except for a slight decrease (80% of control; $0.05 < P < 0.1$) during the first week. An increase in the dietary $PdCl_2$ concentration to 29.8 mmoles (5.38 g salt)/kg feed markedly decreased the weight gain of the

Pd-treated rats for each of the first three weeks on the diet but not during the fourth week (Table 2); the weight gain for the full 4-week interval (172 $\pm$ 12 g, S.D.) was 74% ($P < 0.05$) of the weight gain by the paired control rats.

PdO, at a dietary level of 29.8 mmoles (3.64 g salt)/kg feed, decreased the weight gain in the Pd-treated rats only during the fourth week of the four-week diet. However, the weight gain during the entire 4-week interval, 201 $\pm$ 7 g (S.D.), was 81% ($P < 0.01$) of the gain by paired controls.

The use of a saturated solution of $PdSO_4$ as the drinking fluid for 4 weeks did not affect the weight gain/rat during any of the 4 weeks. Likewise, a dietary level of 5.9 mmoles (1.19 g salt)/kg feed did not affect the weight gain/week during any of the 4 weeks on the diet. However, $PdSO_4$ at a level of 29.8 mmoles (6.03 g salt)/kg feed decreased the weight gain of the Pd-treated rats to 54% ($P < 0.01$) of control for the entire 4-week interval of the diet.

The addition of $PtCl_4$ to the drinking fluid (1.63 mmoles (550 mg salt)/liter) decreased weight gain/rat by 15% ($P < 0.01$) during the first week but did not affect the weight gain during each of the remaining three weeks on the diet (Table 2). When the $PtCl_4$ is added to the drinking fluid at a concentration of 0.54 mM (183 mg salt/liter), the Pt-treated rats showed normal weight gain throughout the 12 weeks on the diet. Likewise, when $PtCl_4$ was added to the feed at the level of 5.9 mmoles (1.98 g salt)/kg feed, normal weight gains were observed through each week of a 4-week schedule. However, at a concentration of $PtCl_4$ of 13.2 mmoles (4.46 g salt)/kg feed, the weight gains by Pt-treated rats were decreased to 27% and 76% of the paired controls during the first week and during a total 4-week interval respectively (Table 2), but the weight gains by Pt-treated rats was normal during the second, third and fourth weeks on the diet.

PtO_2, an "insoluble" salt, had no effect on weight gain during each of 4 weeks when present in the feed at a level of 29.8 mmoles (6.76 g salt)/kg feed. $Pt(SO_4)_2.4H_2O$, at a concentration of 1.63 mmoles (750 mg salt)/liter in the drinking fluid, decreased the weight gain by Pt-treated rats during a one-week diet (Table 2). When $Pt(SO_4)_2.4H_2O$ was included in the feed at a level of 5.9 mmoles (2.70 g salt)/kg feed, the weight gain of Pt-treated rats was decreased by 15-18% during the first, second ($P < 0.01$), and third ($P < 0.05$) week of the treatment (Table 2).

Organ weights. The weight of five tissues (liver, kidney, spleen, heart and testis) are presented in Table 3. The data are expressed as the percentage of the expected weight (based on the body weight of rats on control and metal-containing diets). The administration of $MnCl_2.4H_2O$ for 13-weeks, at the doses used, did not affect the weights of any of the five tissues.

Dietary $PbCl_2$ did not consistently affect the weights of any of the tissues except kidney (Table 3). In all five dietary schedules used, $PbCl_2$ increased the weight of kidney (expressed as a percentage of the kidney weight expected of rats of equal body weight) and the increases often exceeded 25% above the expected weight. As discussed later, the kidney enlargement in Pb-treated rats has been reported previously in work by others.

In each of two experiments in which anhydrous $PdCl_2$ was added to the feed at the level of 13.2 mmoles/kg feed, there was a reduction in the weights of the liver, kidney and spleen but not in the heart or testis. The combined data of the two experiments (8 rats) are presented in Table 2. Upon feeding other Pd-containing salts, however, no consistent pattern of changes in organ weights was observed. In a single experiment (4 control and 4 Pd-treated rats), PdO caused a decrease in the heart weight.

The dietary administration of Pt^{4+} salts did not markedly change the weights of any organs. The administration of $Pt(SO_4)_2.4H_2O$ (1 week; 1.6 mmoles/liter drinking fluid) did decrease the liver weight and the administration of $PtCl_4$ (4 weeks; 1.6 mmoles/liter drinking fluid) increased the kidney weight. Under most of the schedules of the dietary administration of Pt^{4+} salts, small increases were noted in the size of the kidney but the increase was statistically significant in only one series (Table 3).

Tissue content of DNA, RNA, and protein. Following the dietary administration of metallic salts, various tissues were analyzed for the content of DNA, RNA, and protein. Rats which had received $PbCl_2$ at a level of 3.7 mmoles/liter drinking fluid for 4 weeks did not show altered DNA, RNA or protein content in liver, kidney or spleen (Table 4). Although in one series of rats there was an apparent decrease in the DNA content of testis of Pb-treated rats, analyses on testis from a second series of animals showed no such decrease.

The administration of Pt^{4+} salts, either as $PtCl_4$ at 13.2 mmoles/kg feed for 4 weeks or as $Pt(SO_4)_2.4H_2O$ at 5.9 mmoles/kg feed for 4 weeks, did not alter the tissue content of DNA, RNA or protein in liver, kidney or spleen (Table 4).

DISCUSSION

On a molar basis, the soluble salts of platinum and palladium are among some of the more toxic metallic salts. It is anticipated that the exposure of populations to these metallic salts will be low due to the relative rarity of the compounds.

The inclusion of the metallic salts in the diets of rats at the doses used in this study commonly resulted in decreased weight gain by the metal-treated rats. In most cases, however, the decreased weight gain was reflected in the decreased feed consumption by these rats.

In a manner similar to the results reported by Hirsch (1973) and by others, the dietary administration of lead salts resulted in enlargement of the kidney. Hirsch showed that the increased weight in the kidney was not due to an increased percentage of water content. The dietary administration of platinum and palladium salts did not bring about major and/or consistent changes in the weights of the five tissues examined. Likewise, dietary treatment with salts of platinum or palladium did not alter the content of DNA, RNA or protein in liver, kidney or spleen (when the content is expressed per gram of wet tissue).

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SECTION III. Table 1.

Lethal Doses of Various Metallic Compounds After Intraperitoneal or Oral Administration in the Rat

Compound	Route	Lethal Dose[b] (mmoles/kg)	Lethal Dose[b] (mg cation/kg)
$MnCl_2.4H_2O$	ip	0.70 (0.61-0.80); 0.56; 0.87	38 (33-44); 31; 48
	oral	7.5 (7.0-8.1); 6.3; 9.0	410 (380-450); 350; 490
$PbCl_2$	ip	8.5 (5.0-14.4); 1.6; 16.8	1760 (1050-3000); 330; 3500
$PdCl_2.2H_2O$	ip	0.57 (0.45-0.72); 0.39; 0.82	60 (48-77); 42, 87
	oral	2.7 (2.2-3.4); 1.56; 4.8	290 (240-360); 166; 520
$PdSO_4$	ip	1.42 (1.11-1.81); --; 1.8	151 (118-193); —; 195
$PtCl_2$	ip	2.5 (1.58-4.0);	490 (310-770);
$PtCl_4$	ip	0.11 (0.09-0.15);	22 (17-29);
	oral	0.70 (0.51-0.96); 0.31; 1.57	136 (99-188); 60; 310
$Pt(SO_4)_2.4H_2O$	ip[c]	0.68 (0.60-0.76); 0.56; 0.82	132 (117-149); 110; 160
	ip[d]	0.3-0.4; 0.2-0.3; 0.4-0.6	59-78; 39-59; 78-117
	oral	2.2 (1.57-3.1); 1.37; 3.5	430 (310-600); 270; 690
$RuCl_3$	oral	3.2 (2.4-4.0); 1.78; 5.4	310 (240-400); 180; 550

[a]Male Sprague-Dawley rats; initial body weight 100-110g. A 14-day observation period was used in lethal dose studies.

[b]Data are given in the following sequence: LD_{50} (and its 95% confidence limits, in parentheses); LD_{10}; LD_{90}

[c]Compound obtained from ICN/K and K Laboratories

[d]Compound obtained from D. F. Goldsmith Chemical and Metal Corporation

SECTION III. Table 2.

Effect Of Dietary Metallic Salts On Weight Gain

Metallic Salt	Group	Dietary Level[a]	Parameter Measured[b]	Week Number 1	2	3	4	1-4
$PbCl_2$	Control	--	W (4)	110	120	89	97	104
	Pb	13.2/kg	W (4)	72**	106ns	85	82	81†
	Control	--	W (12)	106	103	99	104	103
	Pb	29.8/kg	W (8)	73**	84*	89ns	93ns	84*
$PdCl_2$	Control	--	W (4)	106	105	105	101	104
	Pd	29.8/kg	W (4)	52**	75*	71**	115ns	77*
PdO	Control	--	W (4)	120	105	95	127	112
	Pd	29.8/kg	W (4)	109ns	101ns	90ns	59**	91**
$PdSO_4$	Control	--	W (4)	121	--	--	--	100
	Pd	29.8/kg	W (4)	103†	--	--	--	54**
$PtCl_4$	Control	--	W (12)[c]	99	109	97	100	100
	Pt	1.63/ℓ	W (8)[c]	84**	103ns	103ns	98ns	94ns
	Control	--	W (4)	103	--	--	--	--
	Pt	2.45/ℓ	W (4)	79**	--	--	--	--
	Control	--	W (8)	103	89	79	96	91
	Pt	13.2/kg	W (8)	21**	64*	77ns	70ns	57**

Table 2 (Continued)

Metallic Salt	Group	Dietary Level[a]	Parameter Measured[b]	Week Number 1	2	3	4	1-4
$Pt(SO_4)_2.4H_2O$	Control	--	W (16)	103	--	--	--	--
	Pt	1.63/ℓ	W (8)	74**	--	--	--	--
	Control	--	W (4)	94	115	105	118	108
	Pt	5.9/kg	W (4)	80^{ns}	98**	87*	115^{ns}	95*

[a]Dietary levels of metallic salts are expressed as mmoles/kg feed or mmoles/liter drinking fluid.

[b]Parameters measured include weight gains (W). Weight gains are expressed as percentage of the weight gains during the corresponding week by all control rats maintained on diets for 4 or more weeks. Weight gains (g/rat/week) (mean ± S.D.) by 120-124 control rats were: week 1, 58 ± 10; 2, 57 ± 12; 3, 56 ± 13; 4, 51 ± 14. Weight gains by controls during weeks 1-4 were 222 ± 34.

[c]Minimum number of values for each time interval.

Statistical analysis (t-test): **, $P < 0.01$; *, $P < 0.05$; †, $0.05 < P < 0.1$; ns, $P > 0.1$.

SECTION III. Table 3.
EFFECT OF DIETARY METALLIC SALTS ON TISSUE WEIGHTS

Metallic Salt	Group, No. of Rats	Dietary Level[a]	Duration (weeks)	Body Weight[b] (g)	Tissue Weight (% of Expected Weight)[c]				
					Liver	Kidney	Spleen	Heart	Testis
$MnCl_2.4H_2O$	Control, 8	--	13	517	92	(106)	92	(97)	94
	Mn, 8	8.3/ℓ	13	520	91	(102)	105	(103)	94
	Control, 4	--	13	578	93	(97)	(90)	(101)	(103)
	Mn, 4	18.6/ℓ	13	511	88	(105)	(87)	(93)+	(95)
$PbCl_2$	Control, 8	--	4	309	104	(95)	(82)	(104)	(102)
	Pb, 8	3.7/ℓ	4	309	107	(101)+	(88)	(108)	(100)
	Control, 4	--	4	344	105	(96)	96	(107)	(101)
	Pb, 4	8.3/ℓ	4	296	102	(112)+	104	(104)	(96)
	Control, 8	--	13	511	89	(103)	(103)	(99)	(100)
	Pb, 8	3.7/ℓ	13	482	98*	(127)**	(106)	(99)	(97)
	Control, 8[d]	--	4	318	103	(107)	(101)	102	(98)
	Pb, 8[d]	13.2/kg	4	286	106	(131)*	(95)	101	(99)
	Control, 8	--	4	297	108	(106)	(114)	99	(96)
	Pb, 8	29.8/kg	4	258*	107	(143)**	(132)	106	(100)*

Table 3 (Continued)

Metallic Salt	Group, No. of Rats	Dietary Level[a]	Duration (weeks)	Body Weight[b] (g)	Tissue Weight (1% of Expected Weight)[c]				
					Liver	Kidney	Spleen	Heart	Testis
$PdCl_2.2H_2O$	Control, 8	--	1	136	85	(97)			
	Pd, 8	(satd. soln.)	1	132	85	(95)			
$PdCl_2$	Control, 8	--	4	335	113	(102)	(134)	(95)	(103)
	Pd, 8	13.2/kg	4	318	101**	(93)**	(89)**	(96)	(104)
	Control, 4	--	4	314	112	(97)	(100)	105	(103)
	Pd, 4	29.8/kg	4	258*	114	(99)	(82)	103	(106)
PdO	Control, 4	--	4	324	104	(98)	(79)	(104)	(97)
	Pd, 4	29.8/kg	4	287**	103	(106)†	(96)	(95)*	(100)
$PdSO_4$	Control, 4	--	4	295	104	(104)	(93)	(98)	(101)
	Pd, 4	5.6/kg	4	318	105	(108)	(101)	(100)	(98)
	Control, 4	--	4	318	102	(128)	(103)	102	(101)
	Pd, 4	29.8/kg	4	238*	88	(124)	(86)	103	(112)†
$PtCl_4$	Control, 4	--	13	547	92	(107)	(92)	(103)	(96)
	Pt, 4	0.54/l	13	538	88	(114)	(94)	(102)	(95)

Table 3 (Continued)

Metallic Salt	Group, No. of Rats	Dietary Level[a]	Duration (weeks)	Body Weight[b] (g)	Tissue Weight (1% of Expected Weight)[c]				
					Liver	Kidney	Spleen	Heart	Testis
$PtCl_4$	Control, 12	--	1	152	89	88	96	97	76
	Pt, 12	1.63/kg	1	145	87	91	91	97	79
	Control, 12	--	4	306	100	(89)	(78)	103	(99)
	Pt, 12	1.63/kg	4	296	100	(95)**	(81)	104	(106)+
	Control, 4	--	4	287	107	(95)	(124)	(101)	(98)
	Pt, 4	5.9/kg	4	279	107	(99)	(112)	(104)	(99)
	Control, 4	--	4	288	116	(117)	105	96	(105)
	Pt, 4	13.2/kg	4	252*	96	(123)	93	101	(106)
PtO_2	Control, 4	--	4	272	102	(108)	96	101	(104)
	Pt, 4	29.8/kg	4	300	105	(115)	92	102	(103)
$Pt(SO_4)_2.4H_2O$	Control, 8	--	1	173	99	(99)	105	107	89
	Pt, 8	1.63/kg	1	149*	88*	(97)	91	93	87
	Control, 4	--	4	328	109	(109)	(104)	101	(99)
	Pt, 4	5.9/kg	4	285**	113	(128)	(120)	100	(103)

Table 3 (Continued)

Statistical analyses (t-test): **, $P < 0.01$; *, $P < 0.05$; †, $0.05 < P < 0.01$; no designation is used were $P > 0.1$.

[a]Dietary levels of metallic salts are expressed as mmoles/liter drinking fluid or mmoles/kg feed.

[b]Body weight and tissue weights were measured after fasting for 14-15 hours.

[c]Expected tissue weights were calculated from the equations given in the MATERIALS AND METHODS, except that values in parentheses were calculated from similar equations calculated only for control rats on the same dietary duration (that is, 1-, 4- or 13-weeks).

[d]Except only 4 values for liver, heart and testis.

SECTION III. Table 4.

EFFECT OF DIETARY METALLIC SALTS ON THE CONCENTRATION OF DNA, RNA AND PROTEIN IN VARIOUS TISSUES

Salt	Group[a]	Dietary Level	Duration (weeks)	Macromolecule[b]	Tissue			
					Liver	Kidney	Spleen	Testis
$PbCl_2$	Control	--	4	DNA	5.19 ±0.69	6.86 ±1.06	40.1 ±1.7	6.06 ±0.45
				RNA	22.7 ±0.9	9.84 ±1.01	18.6 ±2.7	8.94 ±0.51
				Protein	298 ±95	92.2 ±29.1	122 ±9	117 ±22
	Pb	3.7/ℓ	4	DNA	4.64 ±1.06	7.05 ±0.47	38.6 ±3.8	5.32* ±0.10
				RNA	20.9 ±2.1	11.90 ±2.10	22.2 ±4.5	9.31 ±1.00
				Protein	279 ±59	90.3 ±18.2	129 ±7	129 ±9
$PbCl_2$	Control	--	4	DNA	--	8.42 ±0.94	38.3 ±6.0	6.62 ±0.52
				RNA	27.3 ±0.7	10.94 ±1.59	23.6 ±1.4	9.90 ±0.59
				Protein	--	137 ±15	109 ±12	63.3 ±10.0
	Pb	3.7/ℓ	4	DNA	--	8.03 ±0.66	35.8 ±12.8	6.67 ±0.27
				RNA	27.1 1.2	11.20 ±0.52	23.7 ±6.3	10.66 ±1.42
				Protein	--	143 ±4	117 ±17	64.6 ±5.8
$PtCl_4$	Control	--	4	DNA	6.82 ±0.72	6.51 ±0.63	28.3 ±3.1	--
				RNA	23.0 ±1.6	8.10 ±0.51	17.7 ±1.3	--
				Protein	188 ±37	113 ±17	103 ±45	--

Table 4 (Continued)

EFFECT OF DIETARY METALLIC SALTS ON THE CONCENTRATION OF DNA, RNA AND PROTEIN IN VARIOUS TISSUES

Salt	Group[a]	Dietary Level	Duration (weeks)	Macromolecule[b]	Tissue			
					Liver	Kidney	Spleen	Testis
$PtCl_4$	Pt	13.2/kg	4	DNA	6.49 ±0.38	6.50 ±0.76	27.4 ±1.6	--
				RNA	21.2 ±2.4	7.99 ±1.02	18.7 ±2.0	--
				Protein	230 ±36	93† ±8	102 ±34	--
$Pt(SO_4)_2$ $.4H_2O$	Control	--	4	DNA	7.64 ±0.52	6.66 ±0.32	39.3 ±7.9	--
				RNA	23.2 ±1.5	9.58 ±0.50	19.2 ±0.9	--
				Protein	164 ±12	111 ±6	114 ±5	--
	Pt	5.9/kg	4	DNA	6.96 ±1.44	7.09 ±0.71	36.4 ±2.1	--
				RNA	21.1† ±1.1	9.40 ±1.30	19.5 ±2.3	--
				Protein	170 ±20	104 ±12	116 ±12	--

Statistical analysis (t-test): *, $P < 0.05$; †, $0.05 < P < 0.1$; no designation is used where $P > 0.1$

[a] 4 rats/group

[b] DNA content is expressed as umoles DNA-nucleotide/g wet tissue; RNA as umoles RNA-nucleotide/g wet tissue; and protein as mg protein/g wet tissue.